Determination of cortisol in serum

A-24.2

Key words

Instrumental HPTLC - quantitative analysis - fluorescence derivatization - densitometry (fluorescence) - antirheumatics - antiallergikum - antiphlogistics - steroids - cortisol

Scope

The cortisol concentration in serum is between 60 µg/L and 250 µg/L. Prechromatographic derivatization increases detection sensitivity. Thus, after chromatography on silica gel with benzene - dioxan - methanol 8:2:1, fluorescence measurement gives a determination limit of less than 40 µg/L in serum. The results correlate well with those found by radioimmunoassay (RIA).

Literature

W. Funk: Fresenius Z. Anal. Chem. **318**, 206-219 (1984)

W. Funk, R. Kerler, E. Boll, V. Daumann: J. Chromatogr. **271**, 349-355 (1981)

R. Kerler, E. Boll: diploma-thesis - Faculty of occupational health, Giessen Polytechnic 1980 and 1981

Advantages of using planar chromatography for this analytical task

- Substantially greater cost effectiveness than RIA, particularly if the daily (weekly) number of such analyses is only small

Chemicals

Standard: Hydrocortisone Merck (24608) = cortisol
ClinElut prepacked columns (No. 1001, Analytichem)
Dichloromethane
Methanol
Ethanol
Benzene
Dioxane (redistilled with TCA and 2.4-dinitrophenyl hydrazine)
Butylhydroxytoluene (antioxidant)
Paraffin oil
n-Hexane
Trichloroacetic acid (TCA)
Dansyl hydrazine

Sample preparation

a) Extraction

- Apply 3 mL serum to a ClinElut column and wait 3 min.
- Elute twice with 6 mL dichloromethane, collect the combined eluate in a 15 mL beaker with a curved rim and evaporate to dryness under nitrogen.

b) Derivatization:

- Dissolve the residue in 200 µL TCA solution (15 mg TCA in 500 mL ethanol) . Add 100 µL hydrazine solution (4 mg dansyl hydrazine dissolved in 20 mL ethanol. This solution is stable for 1 week if stored in the dark), and incubate the mixture 30 min in the dark at room temperature.
- Evaporate to dryness in a stream of nitrogen, and dissolve the residue in 5 mL ethanol.

Standard solution

- Dissolve 60 mg hydrocortisone in 10 mL dioxane.
- 10 µL of this solution make up to 100 mL with dioxane.
- Evaporate 5 mL to dryness under nitrogen.
- Dissolve the residue in 200 µL TCA solution, add 100 µL dansyl hydrazine solution.
- Incubate 30 min in the dark and evaporate to dryness under nitrogen.
- Dissolve the residue in 5 mL ethanol (600 pg/µL).

Layer

HPTLC plates Merck silica gel 60 F_{254}, 20 x 10 cm, prewashed by blank chromatography with methanol, then dried for 20 min at 110°C.

Sample application

With CAMAG Linomat as 7 mm bands, track distance 5 mm, distance from left edge 15 mm, distance from lower edge 8 mm, delivery rate 5 s/µL = 15 applications per plate.

Application pattern:

S1	U1	S2	U2	S3	U3	S4	U4	S1	U1	...	
1	2	2	2	3	2	4	2	1	2	...	µL/band

S1-4 = standards in different concentrations; U 1-4 = unknowns

Alternatively the Automatic TLC Sampler can be used.

Chromatography

In CAMAG Horizontal Developing chamber with benzene - dioxane - methanol 8:2:1 (0.1% butyl hydroxytoluene added as antioxidant).

The relative air humidity must not be higher than 47%. If it is above that level, the plate is preconditioned for 30 min at 42% (286 mL conc. sulfuric acid in 500 mL water).

Running time 15 min, migration distance 50 mm, Rf about 0.4.

Fluorescence intensification

The wet plate is immersed for 1 s with CAMAG Chromatogram Immersion Device in a solution of paraffin oil - n-hexane 5:1. This increases the fluorescence intensity by a factor of 10 and stabilizes it for 2 h.

Densitometric evaluation

With CAMAG TLC Scanner and CATS evaluation software; scanning by fluorescence at 366/>400 nm.

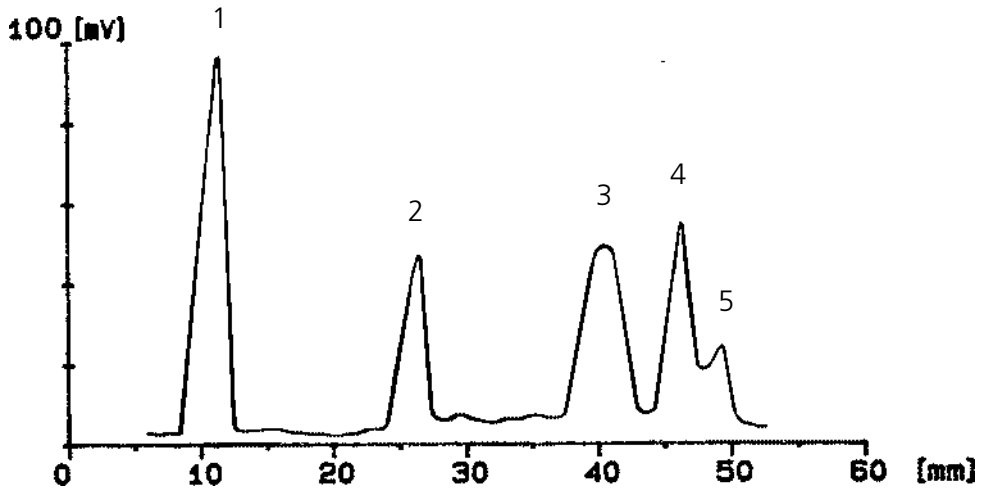

Fig. 1 Densitogram of a sample containing cortisol dansyl hydrazone (2), by-products (3-5) and start zone (1).

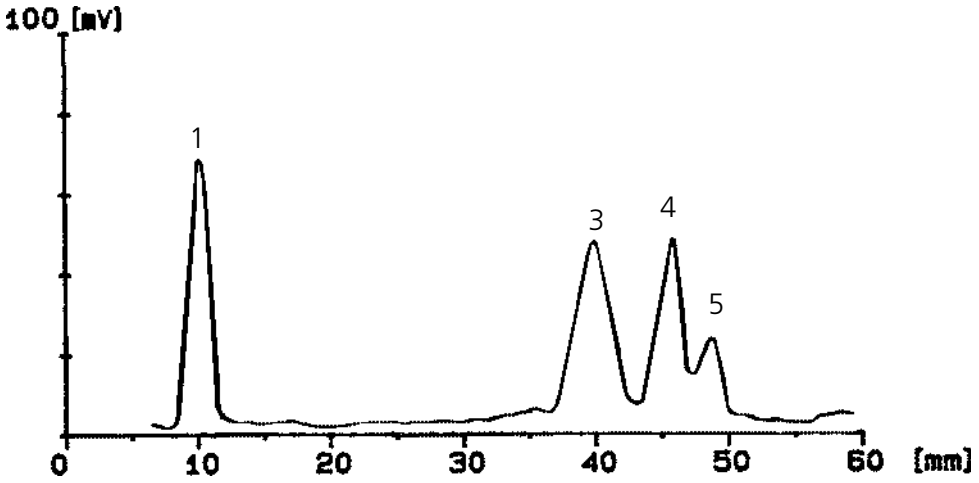

Fig. 2 Densitogram of blank sample (same number as fig. 1)

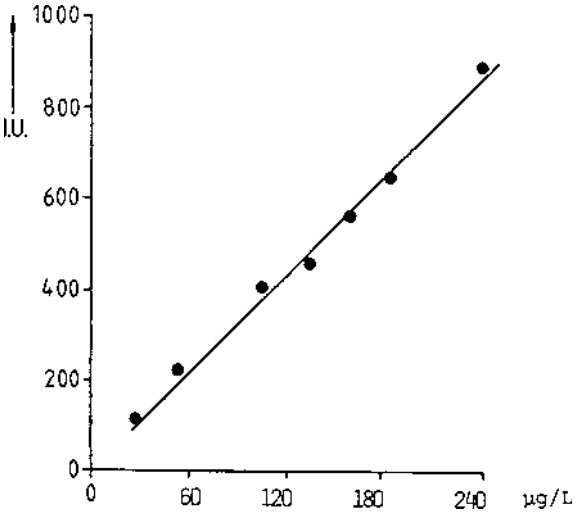

Fig. 3

Linear regression of cortisol dansyl hydrazone

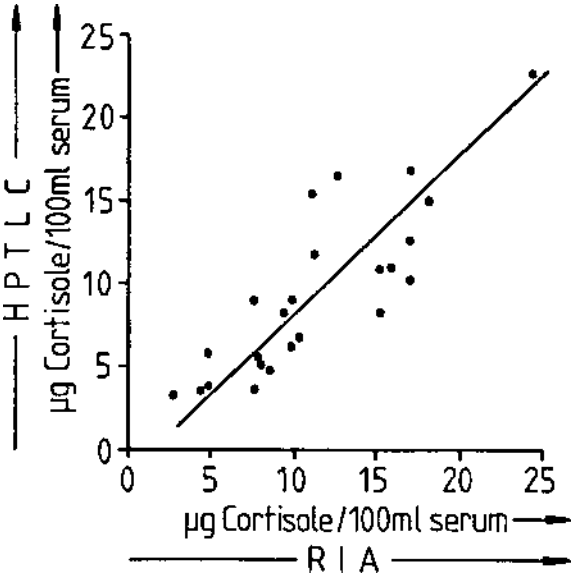

Fig. 4

Results obtained from quantitative HPTLC determination of 24 different sera are compared with those found by RIA.

Orthogonal regression yields a correlation coefficient of 0.845, confirming the comparability of the two analytical procedures.